

# Quality assurance in extemporaneously compounded formulations: a titration method for ursodeoxycholic acid

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- **OBJECTIVE** — To validate an acid-base titration method, adopted from the British Pharmacopoeia, for analysis of ursodeoxycholic acid (UA) in extemporaneously compounded formulations.
- **METHODS** — Two different extemporaneously compounded formulations containing UA 15mg/ml, prepared from both pure drug and Actigall capsules were compared. A series of titrations was carried out to validate the titration method, as per International Conference on Harmonisation (ICH) guidelines. The titration method was used to analyse the stability of the UA suspensions stored for six weeks under ICH accelerated stability conditions.
- **RESULTS** — The titration method was found to be specific and accurate. Differences between the initial drug concentration of the two suspensions and the concentration after six weeks at accelerated stability conditions were statistically insignificant, indicating stability of the formulations.
- **CONCLUSION** — A simple acid-base titration method adopted from the British Pharmacopoeia has been validated to determine UA content in extemporaneously compounded suspensions, and can be recommended for quality assurance testing in hospital pharmacy.

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**E**xtemporaneously compounded medicines are required for specific patients when a commercial pharmaceutical product is not available.<sup>1</sup> Within the pharmaceutical manufacturing industry, quality, safety and efficacy are enforced by regulatory legislation covering good manufacturing practice, good laboratory practice, and good clinical practice. In contrast, the responsibility for acceptable standards for compounding “off-label” medicines lies with the pharmacist. Extemporaneous products are often assigned short “beyond-use” dates when supporting stability data are not available.<sup>2</sup>

High-performance liquid chromatography (HPLC), is commonly used for stability studies in the pharmaceutical industry, but may not be freely available in hospital pharmacy. The aim of this study was to validate an acid-base titration method of analysis which could be used in a hospital pharmacy. Ursodeoxycholic acid (UA) was selected as the drug of choice, because the analytical results could be compared with literature and the active ingredient could be purchased for analytical purposes.

UA is a bile acid present in low concentrations in humans. It is used in the dissolution of cholesterol-rich gall stones in patients for whom surgery is not possible or is undesirable. It is also used to treat diseases of the liver including biliary cirrhosis, primary sclerosing cholangitis and cystic fibrosis related cholestasis.<sup>3</sup>

The recommended dose varies from 8–15mg/kg daily for adults and 15–20mg/kg for children, given in divided doses. If patients require liquid dosage forms due to swallowing difficulties or age the drug needs to be reformulated.<sup>4</sup> UA is almost insoluble in water so can be compounded in the form of a suspension.<sup>5,6</sup> The formulation (15mg/ml) that is currently compounded in a children’s hospital in New Zealand involves dispersing the contents of a UA capsule in 10ml Ora-Sweet (sweetening agent), and adding an equal volume of Ora-Plus (suspending agent). The product is assigned a beyond-use date of one month if stored at 2–8°C.<sup>7</sup>

The stability of UA in different suspension formulations is well documented.<sup>6,7,8</sup> However, these stability studies were all carried out using HPLC techniques. There is a need for an easy and accurate analytical method for determining drug content in extemporaneously compounded formulations.

## Method

**Materials** Pure UA was procured from Spectrum Chemical (New Brunswick, New Jersey, US). Actigall 300mg capsules (the only UA preparation available in New Zealand) were procured from Novartis. Ethanol 96 per cent (analytical grade), sodium hydroxide pellets (reagent grade) and phenolphthalein were obtained from Scharlau Chemie (Spain). Ora-Sweet (sugar-free syrup) and Ora-Plus (suspending agent) were purchased from local distributors. These products are formulated by Paddock Laboratories, Minnesota, US (further information available at [www.paddocklabs.com](http://www.paddocklabs.com)). Sodium hydroxide solution 0.1M was prepared for the studies. Phenolphthalein solution was prepared by dissolving 0.25g of phenolphthalein in 25ml ethanol. Equipment used in the studies were conical flasks, burettes, pipettes (20ml and 5ml), Gilson pipettes (100–1,000µl), beakers (50ml and 150ml), and volumetric flasks (25ml, 100ml, 1L and 2L).

## Preparation of the formulations

### Standard drug solution

Pure UA (175mg) was dissolved in 25 ml of ethanol (96 per cent v/v) previously neutralised with 0.1ml phenolphthalein solution. Distilled water (25ml) was then added to the solution.

### Suspension A: UA 15mg/ml

A suspension containing UA 15mg/ml was prepared using the contents of 50 Actigall capsules (300mg UA per capsule). The suspending agent (0.05 per cent w/v) and glycerol (10 per cent v/v) were mixed well in a porcelain mortar. For intellectual property reasons the suspending agent is not disclosed in this paper (the formula has been submitted

to a pharmaceutical manufacturing company). Sucrose (25 per cent w/v) was dissolved in 300ml distilled water followed by methylparaben (0.15 per cent w/v). This solution was then gradually added to the mixture in the mortar and mixed well. The contents of the UA capsules (1.5 per cent w/v) were added and mixed well. This mixture was transferred to a 1L volumetric flask, with thorough rinsing of the contents of the mortar into the flask. Lemon flavouring (1 per cent w/v) was added. The resultant suspension was stirred with a glass rod and made up to volume (1L) with distilled water. Quantities of 50ml were transferred into amber glass bottles using a glass pipette and labelled appropriately for the stability studies.

#### Suspension B: UA 15mg/ml

The contents of 50 Actigall capsules (300mg UA per capsule) were mixed with a small amount of Ora-Sweet and Ora-Plus (50 per cent w/v) to make a paste. This paste was then transferred into a measuring cylinder by rinsing with Ora-Plus. The suspension was stirred well and made up to volume (1L) with Ora-Sweet. Quantities of 50ml were then transferred into amber glass bottles using a glass pipette and labelled for stability studies.

Formulations containing pure UA and formulations without UA were prepared in a similar manner by using pure UA instead of Actigall capsules, or without adding any UA, respectively.

**Validation of titration method for determining drug concentration** A simple acid-base titration method adopted from the British Pharmacopoeia was used

**Table 1: Experimental values obtained for method validation of acid-base titration for ursodeoxycholic acid (UA)**

Analyte/formulation	Drug amount(mg)		Recovery (%)	Precision (%RSD)
	Theoretical	Experimental		
UA (pure drug)	175	181.18	103.53	5.56
UA in marketed capsules (Actigall)	300	288.56	96.19	0.68
Suspension A 15mg/ml UA (pure drug)	175	167.18	95.53	7.51
Suspension B 15mg/ml UA (pure drug)	175	168.67	96.38	2.24
Suspension A 15mg/ml UA (Actigall)	175	170.19	97.25	1.43
Suspension B 15mg/ml UA (Actigall)	175	171.37	97.93	0.35

RSD = relative standard deviation

for the quantitative analysis of UA in the suspensions.<sup>9</sup> A series of titrations was carried out in order to validate the method, as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures.

To establish the specificity of the method, the assay was performed on the following:

- Suspension A containing 15mg/ml UA prepared from Actigall and from pure UA
- Suspension A without any active ingredient UA
- Suspension B containing 15mg/ml UA prepared from Actigall and from pure UA
- Suspension B without any active ingredient UA

The analyses were conducted on the same day for each of the formulations for accuracy and precision. The accuracy of the

titration method was determined by analysing samples of pure UA and Actigall capsules containing known amounts of drug. Precision was determined by repeatability of results. The assay was conducted in triplicate and repeatability was evaluated by assaying samples of the same concentration on the same day.

**Application of the titration method to determine stability** Suspensions A and B prepared from Actigall capsules (as described earlier) were stored under ICH accelerated stability conditions (40C ±2C and 75 ±5 per cent relative humidity) for six weeks. The drug concentration of samples was determined using the titration method. Each sample was prepared in triplicate.

## Results

**Validation of titration method for determining drug concentration** Results for the validation studies are shown in Table 1. The specificity of the titration method was established for Suspensions A and B with and without UA. The discrimination of the method was established by subtracting the results of suspensions without UA from suspensions containing UA. In addition, the results were confirmed for suspensions formulated from both pure drug and Actigall capsules.

The percentage recovery of the method for pure drug and marketed capsules was found to be 100 ± 4 per cent. Mean percentage recovery of UA from the formulation made in the laboratory was found to be within the acceptable range of ±5 per cent (Table 1). Precision was determined by repeatability and expressed as percentage relative standard deviation.

**Application of the titration method to determine stability** Concentration of suspensions A and B at six weeks were both maintained above 90 per cent of the initial UA concentration (see Figure 1). Differences between the assay content of UA in these two suspensions were not found

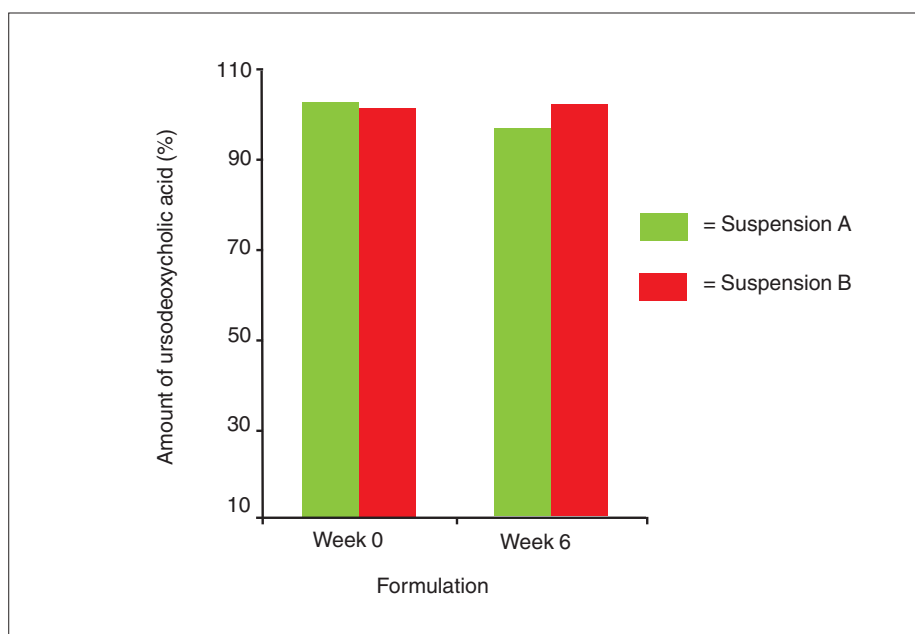


Figure 1: Stability studies of suspensions A and B prepared from Actigall capsules stored at 40C ± 2C and 75±5 per cent relative humidity (concentration expressed as a percentage of initial concentration)

to be statistically significant (Student's *t*-test  $P > 0.05$ , 95 per cent confidence interval) indicating stability of the formulations under the specified conditions.

## Discussion

The method validation and results of the stability studies show the titration method to be specific and accurate. The low standard deviation associated with the stability studies confirmed the accuracy and precision of the method for UA analysis in formulations. The stability of Suspension A at 42 days is in good agreement with previous findings reporting 60 day stability of UA 25mg/ml<sup>5</sup> and 90 day stability of UA 50mg/ml<sup>6</sup> in extemporaneously compounded formulations.

This method requires simple and readily accessible equipment and can be conducted using simple laboratory analytical techniques.

**Limitations** The titration method relies on abrupt endpoint determination (change in colour of the solution being analysed). An inaccuracy in determining the endpoint may result in erroneous calculation of drug concentration.

**Recommendations** The use of acid-base titration methods for determining the concentration of drugs commonly

compounded in hospitals is recommended as part of quality assurance procedures.

## Conclusion

A simple acid-base titration method adopted from the British Pharmacopoeia was used to determine UA content from extemporaneously compounded suspensions. Method validation was performed as per ICH guidelines for specificity, accuracy and precision. The method was found to be simple, rapid and accurate. It was used successfully to determine drug concentration for stability studies. The acid-base titration method can be used effectively in a hospital pharmacy for analysis of UA in extemporaneously compounded formulations.

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